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Award Number:
W81XWH-08-1-0425

TITLE:
Induction and Acceleration of Mammary Tumors by Activated P13 Kinase

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REPORT DATE:
July 2009

TYPE OF REPORT:
annual summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
14-JUL-2009	Annual Summary	15 June 2008-14 JUN 2009

4. TITLE AND SUBTITLE
Induction and Acceleration of Mammary Tumors by Activated P13 Kinase

5a. CONTRACT NUMBER
W81XWH-08-1-0425

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
W91ZSQ7342N688

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
US Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

During this time period the necessary retroviral vectors were developed, used to transduce mammary epithelial cells, and these transduced cells were transferred into the cleared fat pad of recipient mice. Although ductal outgrowth occurred in the cleared fat pads indicating that the transplant was successful, the number of GFP positive ducts that grew out was very low. This was surprising given that a high proportion (greater than 80%) of the mammary epithelial cels were GFP-positive after infection. It is not clear whether the engraftment of the transduced cells was poor, of the outgrowth of these cells was diminished due to high expression of the transgene. Further studies related to these points were curtained due to the departure of the graduate student from graduate school.

15. SUBJECT TERMS
Phosphatidylinositol 3'-kinase, mutation, breat cancer, mouse models

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	4	19b. TELEPHONE NUMBER *(include area code)*

Statement of Work

Training Plan:

Attend weekly journal club—DONE
Attend weekly seminars—DONE
Participate in AACR's pathobiology Cancer Workshop—NOT COMPLETED
Attend PPG monthly meetings and retreat—DONE
Present research at national/international research conferences—NOT COMPLETED

Research Plan:

Task 1: Determine whether the activating mutants of PI3 Kinase induce mammary tumorigenesis invivo.(Months 1-18)

a. Obtain PI3K mutant clones—DONE
b. Insert PI3K mutants into pMIG vectors—DONE
c. Obtain Bosc23 cells and pCL-Eco plasmid—DONE
d. Infect Bosc23 cells with wildtype and mutant PI3K, and vector alone—DONE
e. Collect supernatant containing infectious virus and filter—DONE
f. Harvest primary MECs—DONE
g. Infect and Transplant MECs encoding PI3K mutants, wildtype PI3K, and vector alone controls into recipient mice—DONE
h. Optimize retroviral infection and transplantation protocols—DONE IN PART
i. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
j. Determine phenotype of transplanted MECs infected with wildtype PI3K—NOT COMPLETED
k. Determine phenotype of transplanted MECs infected with mutant PI3K—NOT COMPLETED
l. Analyze tumors, if found, for protein expression levels—NOT COMPLETED
m. Quantitate proliferation and apoptosis of tumors—NOT COMPLETED
n. Analyze activation of signaling molecules downstream of PI3K—NOT COMPLETED

Task 2: Determine whether the activating mutants of PI3 Kinase accelerate mammary tumorigenesis induced by HER2/Neu in-vivo. (Months 12-30)

a. Infect Bosc23 cells with wildtype and mutant PI3K, and vector alone—DONE
b. Collect supernatant containing infectious virus and filter—DONE
c. Obtain MECs from MMTV-c-Neu mice—NOT COMPLETED
d. Infect and Transplant MECs with PI3K mutants, wildtype PI3K, and vector alone controls—NOT COMPLETED
e. Visualize for successful infection of MECs—NOT COMPLETED
f. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
g. Evaluate kinetics of tumor formation—NOT COMPLETED
h. Analyze tumors for differences in histology, protein expression, and metabolism when compared to MMTV-c-Neu control tumors—NOT COMPLETED
i. Quantitate proliferation and apoptosis of tumors—NOT COMPLETED
j. Analyze activation of signaling molecules downstream of PI3K and c-Neu—NOT COMPLETED

Task 3: Determine if the effects of activated PI3 Kinase on mammary tumorigenesis are dependent upon Akt. (Months 20-36)

a. Infect Bosc23 cells with wildtype PI3K and c-Neu*—NOT COMPLETED
b. Infect Bosc23 cells with mutated PI3K and c-Neu*—NOT COMPLETED
c. Infect Bosc23 cells with vector alone—NOT COMPLETED
d. Collect supernatant containing infectious virus and filter—NOT COMPLETED
e. Obtain MECs from Akt 1 KO mice—NOT COMPLETED

f. Obtain MECs from FVB mice (Akt +/+)—NOT COMPLETED
g. Infect MECs (Akt KO and Akt +/+) with collected virus—NOT COMPLETED
a. Transplant infected MECs into recipient mice—NOT COMPLETED
b. Evaluate mammary gland repopulation and ductal formation—NOT COMPLETED
c. Monitor mice for health and tumor growth—NOT COMPLETED
d. If tumors are found; analyze protein expression levels—NOT COMPLETED
e. Quantitate proliferation and apoptosis of tumors, if found—NOT COMPLETED
f. Analyze activation of signaling molecules downstream of PI3K and c-Neu*—NOT COMPLETED

*If PI3K is unable to form tumors without the addition of c-Neu